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Permeability Barrier Disruption Increases the Level of Serine Palmitoyltransferase in Human Epidermis

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Sphingolipids play an important role in the homeostasis and barrier function of human stratum corneum. A disturbance of sphingolipid formation is supposed to be a crucial factor for the increased transepidermal water loss in common skin diseases like atopic eczema or psoriasis. The key enzyme for *de novo* sphingolipid synthesis is serine palmitoyltransferase, which consists of two different subunits, named LCB1 and LCB2 proteins. In order to investigate the induction of LCB2 synthesis in human epidermis, skin barrier disruption was performed by tape stripping on the forearm of healthy volunteers enough to obtain a 3–4-fold increase in transepidermal water loss. Skin punch biopsies were taken before and 0.5, 2, 4, and 8 h after tape stripping by each volunteer to measure LCB2 at the mRNA level. Additional biopsies taken before and 12 h after tape stripping were used to evaluate LCB2 at the protein level. Our results show that 0.5 and 2 h after tape stripping the LCB2 mRNA expression was decreased compared to control in all cases. A significant increase in LCB2 mRNA expression was detectable 4 h after barrier disruption, with individual variations; no further increase was detectable 8 h after tape stripping. Immunohistochemical analysis 12 h after barrier disruption showed increased LCB2 immunolocalization in the inner epidermis, whereas in the outer epidermis it was similar to control. LCB2 mRNA expression preceded the expression of the corresponding protein by 4–8 h. Our findings support the concept that an increase in transepidermal water loss is an obligatory trigger for the upregulation of serine palmitoyltransferase mRNA expression in humans. ***Key words: epidermal lipids/serine palmitoyltransferase/skin barrier disruption/TEWL. J Invest Dermatol 119:1048–1052, 2002***

The stratum corneum provides protection of the underlying skin against environmental noxious influences. At the same time it builds a barrier against transepidermal water loss (TEWL) from the underlying structures to the outside and has been characterized as a dynamic and metabolically interactive tissue. Its architecture has been described best by the "bricks and mortar" model (Elias, 1983) in which epidermal lipids represent the most important component in the formation of the cutaneous permeability barrier. Mainly composed of cholesterol, ceramides, and free fatty acids, they form a multilamellar bilayer structure, which regulates barrier function (Elias, 1981; Lampe *et al*, 1983; Abraham *et al*, 1987; Downing *et al*, 1987; Grubauer *et al*, 1989a; Holleran *et al*, 1995). Ceramides in particular have been shown to have a distinctive role in epidermal permeability barrier homeostasis (Holleran *et al*, 1991b).

The enzymes involved in the synthesis of stratum corneum lipids have been well characterized. Serine palmitoyltransferase (SPT) is the key enzyme for *de novo* sphingolipid synthesis (Holleran *et al*, 1991a). Additionally, there are two pathways for ceramide generation from glucosyl ceramides and from sphingomyelin, catalyzed by β-glucocerebrosidase and by sphingomyelinase, respectively (Holleran *et al*, 1994; Jensen *et al*, 1999). One important signal for the upregulation of skin lipid enzymes is barrier disruption manifested by an increase in TEWL (Grubauer *et al*, 1987; 1989b). The mRNA levels of the most important enzymes like HMG-CoA reductase, HMG-CoA synthase, farnesyl pyrophosphate, and squalene synthase for cholesterol synthesis, acetyl-CoA carboxylase and fatty acid synthase for fatty acid synthesis, as well as SPT for *de novo* sphingolipid synthesis increase after acute barrier disruption as has been shown in hairless mice (Harris *et al*, 1997). Other studies in hairless mice have demonstrated a delayed increase in epidermal sphingolipid synthesis in response to skin barrier abrogation, which contrasts with the immediate response of cholesterol and fatty acid synthesis and confirmed the role of sphingolipids for the maintenance of the epidermal permeability barrier (Holleran *et al*, 1991a; 1991b). No data have been published on the regulation of gene expression of these enzymes in human skin. Mammalian SPT comprises a complex of two subunits named LCB1 and LCB2, with 30% amino acid identity. LCB2 genes have been shown to encode SPT (Weiss and Stoffel, 1997). To evaluate the effect of skin barrier disruption followed by an increase in TEWL on LCB2 mRNA and protein expression in human epidermis we quantified the LCB2 reverse transcriptase polymerase chain reaction (RT-PCR) products and we localized LCB2 protein before and at different time points after barrier disruption.

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Abbreviations: SPT, serine palmitoyltransferase; TEWL, transepidermal water loss.

MATERIALS AND METHODS

Source of tissue The skin of the solar side of the forearm of six healthy (no skin disease and no personal history of atopy) young men (mean age 32±3 y), who had been told neither to apply topical preparations nor to wash the site of investigation 24 h prior to examination, was tape-stripped using cellophane tape (tesa-film, Beiersdorf, Hamburg, Germany). Tape stripping was performed in four areas of approximately 4 cm^2 each, enough to increase the TEWL by three to four times. TEWL was measured at 20°C in a climatized closed room with a relative humidity of 40% using an Evaporimeter (EP1, Servo Medical, Stockholm, Sweden). Three millimeter punch biopsies were taken from each area 0.5, 2, 4, and 8 h after tape stripping. An additional 3 mm punch biopsy was taken without tape stripping and served as control. Immediately after removal, the tissue biopsies were transported in Dulbecco's modified Eagle's medium for further processing.

Preparation of the epidermis The biopsies were washed in phosphate-buffered saline (PBS) and digested in 0.5% dispase solution (Roche Diagnostics, Mannheim, Germany) for 2 h at 37°C. Then the epidermis was mechanically separated, frozen in liquid nitrogen, and stored at −70°C.

Isolation of mRNA The epidermis was harvested in solution buffer (QuickPrep® mRNA buffer, Pharmacia, Uppsala, Sweden) and sonicated for 10 s to destroy the cell membranes without degrading the nucleic acids. Further isolation was performed in three steps following the supplier's protocol (Pharmacia). After ethanol precipitation the harvested mRNA was diluted in 50 µl H_2O aliquots.

Deoxyribonuclease (DNAse) digestion To avoid DNA contamination the mRNA was digested with 10 U DNAse (Roche Diagnostics) with the addition of 10 U RNAse inhibitor (Roche Diagnostics) followed by phenol/chloroform extraction and ethanol precipitation.

RT-PCR For reverse transcription mRNA probes were diluted in 11 µl H_2O and heated together with 0.5 µg oligo$(dt)_{12–18}$ primer at 70°C for 10 min. The reverse transcription reaction was performed at 42°C in 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01 M dithiothreitol, 1 mM dNTP mix, and 200 U SUPERSCRIPT II reverse transcriptase (Gibco BRL, Eggenstein, Germany) for 60 min and stopped at 70°C for 15 min. To nick the mRNA strand, the sample was incubated with 2 U RNAse H (Gibco BRL) for 20 min at 37°C.

For each experiment, primer concentrations, RNA amounts, and PCR cycles were titrated to establish standard conditions. The validity of quantitative comparisons of PCR products was insured by using a number of PCR cycles mapped to within the linear curve. For PCR 1–3 µl of the sample was incubated in 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTP mix, and 0.2 pmol of the chosen primer. The thermal profile used on a Hybaid Limited TouchDown™ Thermal Cycler (Hybaid, Middlesex, U.K.) consisted of denaturation at 94°C for 45 s, annealing at 53°C for 30 s, and an extension temperature of 72°C for 1 min for 32 cycles. With optimized conditions, the PCR runs were repeated on the same RNA samples to assure the reproducibility of the data. Previous studies showed that there is a paucity of low density lipoprotein receptors expressed in human epidermis (Ponec *et al*, 1983; Williams *et al*, 1987). In order to exclude the presence of nonspecific mRNA in the epidermis, RT-PCR for low density lipoprotein receptor (LDLR) was performed on the same tissue biopsies before barrier disruption and using the same conditions as for LCB2.

Oligonucleotides used for PCR were as follows: LCB2 (forward primer 5′-GCTATTGAAAGATGCCATTG-3′, reverse primer 5′-GATCTGCTC-CACTACAGG-3′) (GenBank accession no. U15555), low density lipoprotein receptor primer (forward primer 5′-CAATGTCTCACCAAGCTCTG-3′, reverse primer 5′-TCTGTCTCGAGGGGTAGCTG-3′) (GenBank accession no. NM000527), as an external negative control, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer as an internal standard (forward primer 5′-GGTGAAGGTCGGAGTCAACGGA-3′, reverse primer 5′-GAGGGATCTCGCTCCTGGAAGA-3′) (GenBank accession no. M33197).

Quantification of RT-PCR products and statistics PCR reactions were separated by gel electrophoresis on 2% agarose gels stained with 0.4 mg per ml ethidium bromide. The gels were visualized over an on-line ultraviolet light source transilluminator (Gel Doc 1000 Video documentation System, Bio-Rad, Hercules, CA). The PCR products of the expected size (387 bp for SPT, 238 bp for LDLR, and 430 bp for GAPDH) were then manually defined and the band intensity was quantified using Quantity One quantitation software (Bio-Rad). The intensities of the PCR bands of LCB2 were expressed normalized to GAPDH. For statistical analysis a *t* test was performed. A p-value below 0.05 was considered to be significant.

Immunohistochemistry For the immunohistochemical study, representative tissue biopsies taken at 12 h after tape stripping and the corresponding controls taken before tape stripping were snap frozen in liquid nitrogen for cryostat sectioning. Frozen tissue samples were submerged in Optimum Cutting Temperature embedding compound (OCT, Tissue-Tek, Miles Laboratory, Elkhart, IN) and cryostat sections (4 µm) were prepared on positively charged polylysine-coated slides and allowed to air-dry. Tissue fixation was obtained with fresh 2% paraformaldehyde (Sigma, Deisenhofen, Germany) in PBS for 5 min and methanol for 10 min at 4°C.

All tissue sections were further processed for indirect immuno-histochemistry with an ABC-based method (Vector Laboratories, Burlingame, CA). After blocking both endogenous biotin binding sites with sequential incubations of avidin and biotin, and endogenous peroxidases with 15 min incubation in methanol/1% H_2O_2, the sections were subjected to overnight incubation at 4°C with primary specific rabbit antimouse LCB2 antibody, or control solutions. Mouse anti-LCB2 antibody was generously donated by Professor Wilhelm Stoffel, University of Koeln, Germany (Weiss and Stoffel, 1997).

The antiserum was diluted 1:200. As a positive control, a monoclonal anticytokeratin PAN antibody (Roche Diagnostics) was used at a concentration of 10 µg per ml. Negative controls included use of buffer alone or dilutions of nonspecific purified rabbit IgG in the primary layer (Vector Laboratories). Antibodies were diluted in 0.1% bovine serum albumin (BSA, Sigma) and 0.1% sodium azide (Roth, Karlsruhe, Germany) in PBS (pH 7.4).

Specific binding was detected using a biotin-conjugated horse antirabbit and antimouse IgG, avidin–biotin peroxidase complex (Vector Laboratories), and a substrate solution of H_2O_2 (0.03%) and diaminobenzidine (Sigma, 2 mg per ml) in 0.5 M Tris-saline, pH 7.6, with 1 M imidazole (Roth) and 0.3% sodium azide (Roth). The slides were then counterstained with hematoxylin, dehydrated through graded ethanols and xylene, mounted, and coverslipped.

RESULTS

The mRNA expression of LCB2 is increased 4 h after tape stripping The separation of LCB2 and GAPDH RT-PCR products by gel electrophoresis and ethidium bromide staining showed an increase in the mRNA expression of LCB2 4 h after barrier disruption (**Fig 1**). The values of LCB2 mRNA expression 0.5 and 2 h after tape stripping were lower compared to control levels in all cases. Eight hours after barrier disruption no further increase in LCB2 mRNA was detected. Interindividual differences in LCB2 mRNA baseline expression were observed. No detectable PCR band was found for low density lipoprotein in control human skin, which confirmed the specificity of the tissue (data not shown).

The semiquantitative assessment of the LCB2 RT-PCR bands obtained 4 h after tape stripping and the respective controls are represented normalized to GAPDH expression (**Fig 2**). We show strong interindividual variations in LCB2 mRNA expression, and in two individuals out of six the expression was particularly high. In all volunteers there was a significant elevation of the median value 4 h after tape stripping (**Fig 3**, $p = 0.005$).

The protein level of LCB2 is increased 12 h after tape stripping All samples investigated showed a very similar pattern of immunoreactivity. In control skin LCB2 protein was localized as spotty reactions throughout all epidermal layers, although most abundantly in the granular-corneocyte transition zone and in the stratum basale (*arrows*) (**Fig 4*a***). Also dermal fibroblasts and inflammatory cells stained with the antibody. Twelve hours after tape stripping LCB2 immunolocalization was increased in the inner epidermis compared to controls. In the granular-corneocyte transition zone the protein level of LCB2 was similar to control level (**Fig 4*b***). The negative control showed no stain **(Fig 4*c*)**.

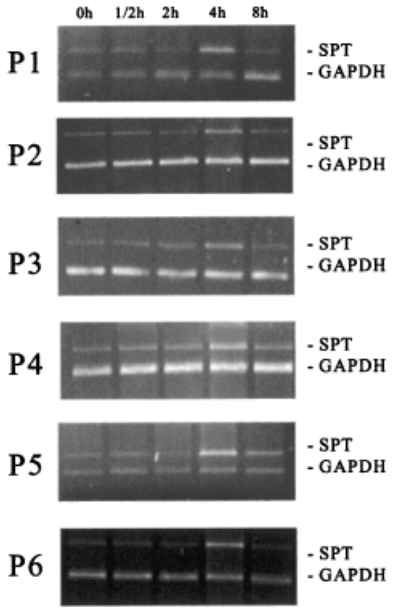

Figure 1. Gel electrophoresis of LCB2 RT-PCR products. Gel electrophoresis of LCB2 (*upper lanes*) and GAPDH (*lower lanes*) in the epidermis of six healthy volunteers (P1–P6) before tape stripping (0 h) and at four different times after tape stripping (0.5, 2, 4, and 8 h). Total cellular RNA was isolated from epidermal specimens. RT-PCR was performed with LCB2 and GAPDH primer, stained with ethidium bromide, and separated on a 2% agarose gel by gel electrophoresis (see *Materials and Methods*)

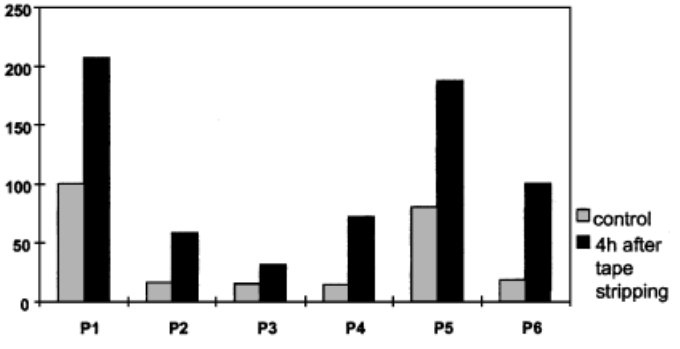

Figure 2. Semiquantitative analysis of LCB2 PCR bands. Relative density levels of LCB2 PCR bands are expressed in percentage of GAPDH. Control levels (*left*) and levels 4 h after tape stripping (*right*) are shown for all volunteers (P1–P6). The densitometric values are obtained from the corresponding ethidium-bromide-stained PCR products presented in **Fig 1**. There is a significant increase in mRNA expression 4 h after barrier disruption in each volunteer.

DISCUSSION

The results of our study show that the LCB2 mRNA level is increased 4 h and the protein level 12 h after acute skin barrier perturbation by tape stripping in humans. The mRNA expression preceded the expression of the protein by 4–8 h. Acute barrier disruption in mammalian skin leads to exocytosis of lamellar bodies at the border between the outer granular layer of the epidermis and the stratum corneum (Menon *et al*, 1992). The lamellar bodies contain the precursor of stratum corneum lipids like glycosphingolipids, phospholipids, and lipid modifiing enzymes (Menon *et al*, 1992). There is evidence that, after accumulated lamellar bodies are used up, fatty acid synthesis starts, which mostly takes place in the basal layers of the epidermis (Monger *et al*,

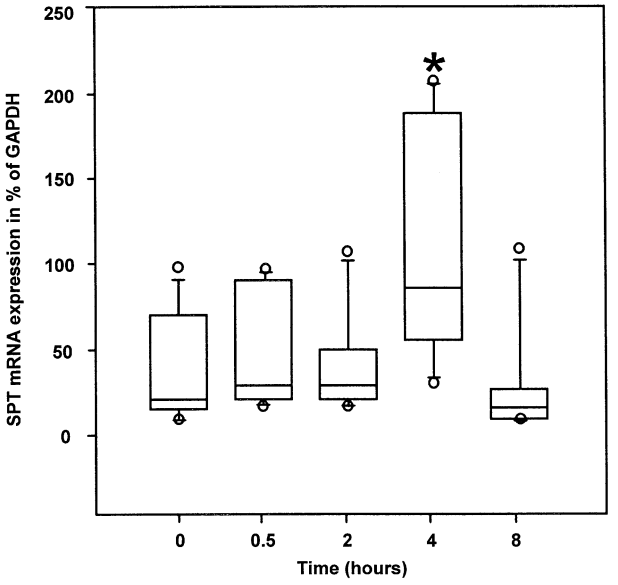

Figure 3. Box plot of the semiquantitative analysis of LCB2 PCR bands. Relative density levels of LCB2 PCR bands are expressed in percentage of GAPDH for each densitometric value obtained from the ethidium-bromide-stained PCR product shown in **Fig 1** at different times (0, before tape stripping; 0.5–8 h after tape stripping). *$p = 0.005$.

1988). Interestingly, we showed by immunostaining increased LCB2 synthesis in the basal epidermal layer. A preliminary study where we evaluated LCB2 immunoreactivity 6 h after tape stripping showed a decrease in LCB2 in the stratum basale, which is then probably replaced by newly synthesized LCB2 12 h after tape stripping. SPT is one of the most important lipid synthesis enzymes involved in skin barrier homeostasis as well as in skin barrier repair and its activity has been localized in both inner and outer epidermis in response to acute and chronic barrier perturbation (Holleran *et al*, 1995). It catalyzes the condensation of serine and palmitoyl-CoA yielding 3-ketosphinganine. The product can be used directly in ceramide synthesis and is consecutively modified to sphingomyelin or glycosylated to gain glycosphingolipids (Kishimoto, 1983). SPT is a highly conserved protein. The identity of the human and mouse fraction of the protein used to produce the antibody anti-LCB2 obtained for our study was 96% (Weiss and Stoffel, 1997). We could then be certain of cross-reactivity on human tissue (Stoffel, personal communication). The two main subunits shown to form SPT with a stoichiometry of 1:1, namely LCB1 and LCB2, have been purified recently by affinity peptide chromatography (Hanada *et al*, 2000). Despite existing discrepancies on the role of the two subunits in determining the enzyme activity, in our study we chose to evaluate the variation in the expression of the LCB2 subunit. Studies on embryonic kidney 293 cells showed that the overexpression of murine LCB2 cDNA was necessary and sufficient to enhance SPT activity in the cells, even without the coexpression of murine LCB1 cDNA (Weiss and Stoffel, 1997).

Direct activating factors for ceramide synthesis are not known but ionic signals or cytokine induction are discussed (Elias, 1996). Previous studies on rodents have shown an increase in SPT mRNA (LCB2 subunit) 4 h after tape stripping (Harris *et al*, 1997) and an increase in SPT activity 5–7 h after barrier disruption, returning to normal after 12 h (Holleran *et al*, 1991a). In our results we showed a similar time curve of LCB2 mRNA expression after barrier disruption, increasing 4 h after tape stripping and returning to basal values after 8 h. The fact that the elevation of the mRNA is not measurable 8 h after the perturbation is remarkable because restoration of 80% of barrier function requires 72–96 h and complete barrier recovery can take up to 14 d (Tagami and Yoshikuni, 1985; Tanaka *et al*, 1997). On the other

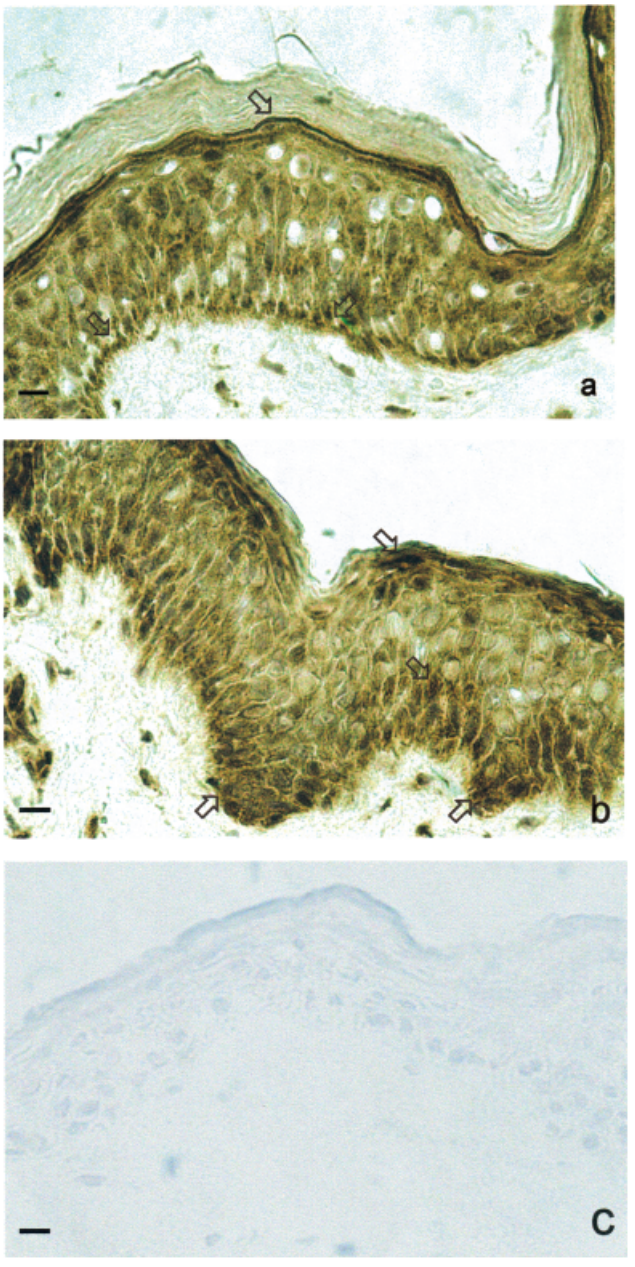

Figure 4. Immunohistochemical localization of LCB2 in normal control skin (*a*), 12 h after tape stripping (*b*), and in nonprimary negative control (*c*). The series represents one individual. The immunohistochemical analysis was performed using the ABC method (see *Materials and Methods*). The LCB2 protein in normal control skin was localized as spotty reactions throughout all epidermal layers, although most abundantly in the granular-corneocyte transition zone and in the stratum basale (*a*, *arrows*). Twelve hours after tape stripping (*b*) LCB2 immunolocalization was increased in the inner epidermis compared to controls (*arrows*). In the granular-corneocyte transition zone the protein level of LCB2 was similar to control. No stain was detected using a nonspecific primary antibody (*c*). *Scale bar:* 30 μm.

hand the basal activity of all lipid modifying enzymes involved in barrier permeability is unusually high (Proksch *et al*, 1990; Holleran *et al*, 1991a; Ottey *et al*, 1995), and small differences of gene expression cannot be captured with the method of semiquantitative RT-PCR.

The increase of SPT after barrier disruption can be inhibited by occlusion of the epidermis, mimicking a reduction in TEWL (Holleran *et al*, 1991b). In two of our volunteers we found a relatively high expression of LCB2. Those volunteers had been examined during the summer, whereas the other biopsies had been taken in autumn and winter. This increased expression corresponds to the finding that total lipid amounts of stratum corneum at air-exposed sites are higher in summertime than in winter (Yoshikawa *et al*, 1994). The higher expression in summertime can be caused either by exposure to ultraviolet irradiation (Wefers *et al*, 1991; Holleran *et al*, 1997; Farrell *et al*, 1998) and/or by the occlusive effect of warm winter clothing. Immunohistochemical analysis revealed a very similar pattern of LCB2 immunoreactivity in different individuals, although the mRNA level showed interindividual variations. This suggests that the excessive mRNA detected in the two aforementioned cases may give similar protein levels to the other cases.

Previous studies had shown that inhibition of SPT results in delay of barrier repair (Holleran *et al*, 1991b). But SPT is not the only enzyme involved in skin barrier function. To guarantee homeostasis in the stratum corneum the combination and coordination of several enzymes of lipid synthesis on the one hand and of lipid metabolism on the other are required (Elias, 1983; 1996; Grubauer *et al*, 1989b; Holleran *et al*, 1993; 1994; 1995; Mao-Qiang *et al*, 1996).

There is evidence that in particular a reduction of enzymes involved in ceramide biosynthesis or metabolism may play a role in frequent skin diseases like atopic eczema and psoriasis. In contrast to hereditary skin alterations such as in Farbers' disease or M. Gaucher, the mechanisms in these diseases are not clear. Several studies have shown that the fraction of ceramides relative to total stratum corneum lipids is diminished in patients with atopic eczema in lesional as well as nonlesional skin (Melnik *et al*, 1988; Hollmann *et al*, 1991; Imokawa *et al*, 1991). These effects have been related to sphingolipid decomposition (Murata *et al*, 1996; Cui *et al*, 1997), but the role of ceramide biosynthesis is not yet clear. Preliminary studies of topical therapy for dermatoses triggered by abnormal barrier function show that application of ceramide-dominant barrier repair lipids improve atopic dermatitis in children (Chamlin *et al*, 2001). In psoriatic lesional skin alterations in ceramide composition are associated with increased levels of TEWL due to a decrease in particular in ceramide 1 (Grice *et al*, 1973; Motta *et al*, 1993; Ghadially *et al*, 1996). We have previously shown altered levels of prosaposin (a key cofactor for sphingolipid degradation) both in lesional and nonlesional skin of patients with psoriasis vulgaris (Alessandrini *et al*, 2001).

Further investigations are necessary to understand the complexity of genetic regulation of skin barrier function and its physiopathologic relevance.

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REFERENCES

Abraham W, Wertz PW, Landmann L, Downing DT: O-acylglucosylceramides and O-acylceramides cause aggregation and stacking of stratum corneum lipid liposomes. *J Invest Dermatol* 88:474A, 1987

Alessandrini F, Stachowitz S, Ring J, Behrendt H: The level of prosaposin is decreased in the skin of patients with psoriasis vulgaris. *J Invest Dermatol* 116:394–400, 2001

Chamlin SL, Frieden IJ, Fowler A, Williams M, Kao J, Sheu M, Elias PM: Ceramide-dominant, barrier-repair lipids improve childhood atopic dermatitis. *Arch Dermatol* 137:1110–1112, 2001

Cui CY, Kusuda S, Seguchi T, Takahashi M, Aisu K, Tezuka T: Decreased level of prosaposin in atopic skin. *J Invest Dermatol* 109:319–323, 1997

Downing DT, Stefwart ME, Wertz PW, Colton SW, Abraham W, Strauss S: Skin lipids: an update. *J Invest Dermatol* 88:2s–6s, 1987

Elias PM: Epidermal lipids, membranes, and keratinization. *Int J Dermatol* 20:1–19, 1981

Elias PM: Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol* 80:44–49, 1983

Elias PM: Stratum corneum architecture, metabolic activity and interactivity with subjacent cell layers. *Exp Dermatol* 5:191–201, 1996

Farrell AM, Uchida Y, Nagiec MM, Harris IR, Dickson RC, Elias PM, Holleran WM: UVB irradiation up-regulates serine palmitoyltransferase in cultured human keratinocytes. *J Lipid Res* 39:2031–2038, 1998

Ghadially R, Reed JT, Elias PM: Stratum corneum structure and function correlates with phenotype in psoriasis. *J Invest Dermatol* 107:558–564, 1996

Grice K, Sattar H, Baker H: The cutaneous barrier to salts and water in psoriasis and in normal skin. *Br J Dermatol* 88:459–463, 1973

Grubauer G, Feingold KR, Elias PM: Relationship of epidermal lipogenesis to cutaneous barrier function. *J Lipid Res* 28:746–752, 1987

Grubauer G, Elias PM, Feingold KR, Harris RM, Elias PM: Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res* 30: 89–96, 1989a

Grubauer G, Elias PM, Feingold KR: Transepidermal water loss: the signal for recovery of barrier structure and function. *J Lipid Res* 30:323–333, 1989b

Hanada K, Hara T, Nishijima M: Purification of the serine palmitoyltransferase complex responsible for sphingoid base synthesis by using affinity peptide chromatography techniques. *J Biol Chem* 275:8409–8415, 2000

Harris IR, Farrell AM, Grunfeld C, Holleran WM, Elias PM, Feingold KR: Permeability barrier disruption coordinately regulates mRNA levels for key enzymes of cholesterol, fatty acid, and ceramide synthesis in the epidermis. *J Invest Dermatol* 109:783–787, 1997

Holleran WM, Feingold KR, Mao-Quiang M, Guo WN, Lee JM, Elias PM: Regulation of epidermal sphingolipid synthesis by permeability barrier function. *J Lipid Res* 32:1151–1158, 1991a

Holleran WM, Man MQ, Goa WM, Menon GK, Elias PM, Feingold KR: Sphingolipids are required for mammalian epidermal barrier function: Inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation. *J Clin Invest* 88:1338–1345, 1991b

Holleran WM, Takagi Y, Menon GK, Legler G, Feingold KR, Elias PM: Processing of glucosylceramides is required for optimal mammalian cutaneous permeability barrier function. *J Clin Invest* 91:1656–1664, 1993

Holleran WM, Takagi Y, Menon GK, Jackson SM, Lee JM, Feingold KR, Elias PM: Permeability barrier requirements regulate epidermal β-glucocerebrosidase. *J Lipid Res* 35:905–912, 1994

Holleran WM, Gao WN, Feingold KR, Elias PM: Localization of epidermal sphingolipid synthesis and serine palmitoyltransferase activity:: alterations imposed by permeability barrier requirements. *J Arch Dermatol Res* 287:254–258, 1995

Holleran WM, Uchida Y, Halkier-Sorensen L, Haratake A, Hara M, Epstein JH, Elias PM: Structural and biochemical basis for the UVB-induced alterations in epidermal barrier function. *Photodermatol Photoimmunol Photomed* 13:117–128, 1997

Hollmann J, Melnik BC, Lee MS, Hoffmann U, Plewig G: Stratum corneum- und Nagellipide bei Patienten mit atopischer Dermatitis. *Hautarzt* 42:302–306, 1991

Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A: Decreased level of ceramides in stratum corneum of atopic dermatitis:: an etiologic factor in atopic dry skin? *J Invest Dermatol* 96:523–526, 1991

Jensen J-M, Schütze S, Förl M, Krönke M, Proksch E: Roles for tumor necrosis factor receptor p55 and sphingomyelinase in repairing the cutaneous permeability barrier. *J Clin Invest* 104:1761–1770, 1999

Kishimoto Y In: Broyer PP, ed. *The Enzymes*, Vol. II. New York: Academic Press, 1983 pp 357–407

Lampe MA, Burlingame AL, Whitney JA, Williams ML, Brown BE, Roitman E, Elias PM: Human stratum corneum lipids: characterization and regional variations. *J Lipid Res* 24:120–130, 1983

Mao-Qiang M, Jain M, Feingold KR, Elias PM: Secretory phospholipase A2 activity is required for permeability barrier homeostasis. *J Invest Dermatol* 106:57–63, 1996

Melnik B, Hollmann J, Plewig G: Decreased stratum corneum ceramides in atopic individuals – a pathobiochemical factor in xerosis? *Br J Dermatol* 119:547–548, 1988

Menon GK, Feingold KR, Elias PM: The lamellar body secretory response to barrier disruption. *J Invest Dermatol* 98:279–289, 1992

Monger DJ, Williams ML, Feingold KR, Brown BE, Elias PM: Localization of sites of lipid biosynthesis in mammalian epidermis. *J Lipid Res* 29:603–612, 1988

Motta S, Monti M, Sesana S, Caputo R, Carelli S, Ghidoni R: Ceramide composition of the psoriatic scale. *Biochim Biophys Acta* 1182:147–151, 1993

Murata Y, Ogata J, Higaki Y, *et al*: Abnormal expression of sphingomyelin acylase in atopic dermatitis: an etiologic factor for ceramide deficiency? *J Invest Dermatol* 106:1242–1249, 1996

Ottey K, Wood LC, Elias PM, Feingold KR: Cutaneous permeability barrier disruption increases fatty acid synthetic enzyme activity in the epidermis of hairless mice. *J Invest Dermatol* 104:401–405, 1995

Ponec M, Havekes L, Kempenaar J, Vermeer BJ: Cultured human skin fibroblasts and keratinocytes: differences in the regulation of cholesterol synthesis. *J Invest Dermatol* 81:125–130, 1983

Proksch E, Elias PM, Feingold KR: Regulation of 3-hydroxy-3-methyl-glutatyl coenzyme A reductase activity in murine epidermis: Modulation of enzyme content and activation state by barrier requirements. *J Clin Invest* 85:874–882, 1990

Tagami H, Yoshikuni K: Interrelationship between water barrier and reservoir functions of pathologic stratum corneum. *Arch Dermatol* 121:642–645, 1985

Tanaka M, Zhen YX, Tagami H: Normal recovery of the stratum corneum barrier function following damage induced by tape stripping in patients with atopic dermatitis. *Br J Dermatol* 136:966–967, 1997

Wefers H, Melnik BC, Flür M, Bluhm C, Lehmann P, Plewig G: Influence of UV irradiation on the composition of human stratum corneum lipids. *J Invest Dermatol* 96:959–962, 1991

Weiss B, Stoffel W: Human and murine serine-palmitoyl-CoA transferase. Cloning, expression and characterization of the key enzyme in sphingolipid synthesis. *Eur J Biochem* 249:239–247, 1997

Williams ML, Rutherford SL, Mommaas-Kenhuis AM, Grayson Vermeer BJ, Elias PM: Free sterol metabolism and low-density lipoprotein receptor expression as differentiation markers in cultured human keratinocytes. *J Cell Physiol* 1332:428–440, 1987

Yoshikawa N, Imokawa G, Akimoto K, Jin K, Higaki Y, Kawashima M: Regional analysis of ceramides within the stratum corneum in relation to seasonal changes. *Dermatology* 188:207–214, 1994